Thiopurines

van Asseldonk, D.P.

2015

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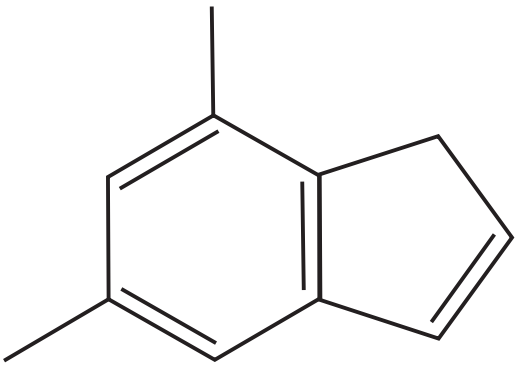

CHAPTER 4

PROLONGED TIOGUANINE THERAPY IS WELL TOLERATED AND SAFE IN THE TREATMENT OF ULCERATIVE COLITIS

Dirk P van Asseldonk
Bindia Jharap
Dirk J Kuik
Nanne K de Boer
Barend D Westerveld
Maurice GVM Russel
Frank JGM Kubben
Adriaan A van Bodegraven
Chris J Mulder

Digestive Liver Disease 2011:43(2):110-5

ABSTRACT

Introduction

Tioguanine has been used in the treatment of inflammatory bowel disease, in particular for those patients who have failed conventional thiopurine therapy. To date, tioguanine has been infrequently studied in ulcerative colitis. The aim of this study is to evaluate tolerability, safety and efficacy of tioguanine in the treatment of ulcerative colitis.

Methods

A database analysis was performed of inflammatory bowel disease patients who had failed conventional thiopurine therapy and been treated with tioguanine. The rates and reasons for treatment failure were assessed. Laboratory values, abdominal ultrasonography, liver biopsy results and rates of endoscopic remission were evaluated.

Results

Forty-six ulcerative colitis patients were included and the median treatment duration was 22 months (range 0.3-72.0). Nine patients failed tioguanine therapy: six due to adverse events and three due to therapy resistance. Concomitant treatment with aminosalicylates protected against tioguanine failure (HR 0.11, 95% CI 0.03-0.48). Where performed, ultrasonography (n=21) revealed no suspected therapy related pathology in all but one patient, in whom hepatomegaly was observed. Liver histology (n=12) predominantly revealed no abnormalities (n=4) or non-specific regeneration (n=4); none showed nodular regenerative hyperplasia. At follow-up, 40% of colonoscopies revealed endoscopic remission as compared with 10% at baseline (p=0.180).

Conclusion

The long-term use of tioguanine appears to be well tolerated and relatively safe in ulcerative colitis patients who have failed conventional thiopurine therapy.

INTRODUCTION

For decades, the thiopurines mercaptopurine (MP) and its pro-drug azathioprine (AZA), have been used in the treatment of inflammatory bowel diseases (IBD). Unfortunately, around 50 percent of patients withdraw from therapy due to the development of adverse effects or therapy resistance[1]. An effective second-line agent in the maintenance treatment of Crohn's disease (CD) is methotrexate (MTX). However, the use of MTX in the treatment of ulcerative colitis (UC) is controversial, and off-label[2]. Although cyclosporine, tacrolimus and infliximab effectively induce remission in UC, only infliximab seems to be an effective alternative to thiopurines as maintenance treatment in a specific and small proportion of patients[3]. Infliximab, however, is associated with well-known and potentially severe adverse events and bears a high economic burden.

Recently, tioguanine (TG) was reintroduced as an alternative oral drug in the treatment of chronic active IBD. Tioguanine is directly metabolized into the putative pharmacologically active 6-thioguanine nucleotides (6-TGN), whereas AZA and MP metabolism involves the production of potentially toxic 6-methylmercaptopurine ribonucleotides (6-MMPR). Although therapeutic results from initial studies showed promise[4,5], an association between the use of TG and the occurrence of nodular regenerative hyperplasia (NRH) has largely discouraged its further use[6,7]. It has been postulated that NRH is a dose or concentration dependent phenomenon, as moderate (lower) dose TG was not related to NRH[8]. Recently, Ansari and colleagues corroborated these findings[9].

Tolerability, safety and efficacy of TG in IBD treatment has primarily been assessed in CD patients, while in UC patients these data are limited. Therefore, we aimed to determine long-term tolerability, safety and efficacy of TG in a Dutch cohort of UC patients previously intolerant of or resistant to conventional thiopurines.

MATERIALS AND METHODS

Patients

A multi-centre cohort study of Dutch IBD patients using TG was conducted in one university hospital and three district hospitals. This study was performed in accordance with the 2008 declaration of Helsinki. All UC patients who initiated TG therapy between 2001 and 2007 were retrieved from a database. Informed consent was obtained from all patients prior to TG treatment.

Study design

Tioguanine was prescribed in a daily dose of approximately 0.3 mg/kg and administered as 18, 21 or 24 mg capsules (generic) or 20 mg tablets (Lanvis®, GlaxoSmithKline, Middlesex, UK). Patient demographics, clinical details, history of prior immunosuppressive therapy, reason for initiating TG therapy and laboratory results were collected from patient records. Patients visited the outpatient clinic on a regular basis for clinical assessment and safety monitoring, in general between two to four visits yearly.

Inclusion and exclusion criteria

Patients were eligible for inclusion if they were diagnosed with UC according to conventional clinical, endoscopic and histological criteria, and required immunosuppressive therapy (chronic active disease and/or corticosteroid dependent) having been proven to be intolerant of or resistant to AZA and/or MP. Resistance to AZA or MP was defined as persisting or deteriorating active disease at six months of therapy despite dose escalation, if appropriate and feasible. Contraindications to receiving TG were: inadequate bone marrow function, active infection, (expected) pregnancy, lactation, and pre-existing liver disease. Notably, not excluded were those patients who had been using cyclosporine, tacrolimus, or infliximab.

Tolerability

Withdrawal of TG was indicated if symptoms were deemed refractory to TG therapy (based on treating physician's global assessment), the development of adverse events or for other miscellaneous reasons. Withdrawal rates and reasons were reviewed both at six months of therapy and at the time of the patient's last evaluation obtained in this study.

Safety monitoring

Laboratory investigations for full blood count, C-reactive protein and liver tests were routinely performed. If performed, red blood cell 6-TGN concentrations were measured after at least four weeks of TG therapy by reversed-phase high-performance liquid chromatography according to the method described by Dervieux and Boulieu[10]. The obtained 6-TGN values were subsequently adjusted to values that would have been obtained by using the method of Lennard et al.[11,12]. In addition to the assessment of platelet counts and liver tests, abdominal ultrasonography was recommended after at least six months of TG therapy to look for signs of hepatotoxicity. All abnormal findings were scored; emphasis was put on signs indicative of portal hypertension, such as splenomegaly and the presence of periportal collateral veins. Liver biopsy was strongly advised at least six months after the initiation of TG therapy according to current guidelines[13], and performed if the patient consented. Specimens were obtained by an ultrasound-guided needle biopsy and immediately fixed in formalin. Subsequently, haematoxylin and eosin, trichrome, and reticuline silver staining was performed. In each hospital liver histology was evaluated by a pathologist with a special interest in liver pathology.

Endoscopy

Endoscopic findings were recorded from the colonoscopy reports of patients who had undergone a colonoscopy both at baseline (up to three months prior to TG initiation) and during follow-up (at least six months after TG initiation). Endoscopic remission was present if there were no signs of active inflammation.

Statistical analysis

The data are tabulated or presented descriptively. The distribution of continuous variables was assessed and log-transformed if skewed. Missing data were checked whether they had occurred at random. Student's T-tests and one-way ANOVA were used where appropriate. Kaplan-Meier analysis was used to illustrate the TG attrition rate and Cox regression analysis was performed to determine the influence of co-treatments. In addition, Wilcoxon signed rank test was performed to compare the rate of endoscopic remission between baseline and follow-up and logistic regression analysis was performed to determine the influence of co-treatments. P values less than 0.05 were considered statistically significant. SPSS 15.0 for Windows (SPSS Inc, Chicago, IL, USA) was used for statistical analysis.

RESULTS

Patients

During the study period, 56 UC patients received TG of whom 46 were included in the analysis. Two patients were excluded due to an unknown TG initiation date, three were excluded as they had no documentation of prior use of AZA and/or MP, and five were excluded as it was unknown whether they had been intolerant of, or resistant to AZA or MP. Of those included, 23 patients were male (median age 43 years, range 19-71) and 23 were female (median age 31 years, range 20-63). Additional demographic data and concomitant therapies are listed in **Table 1** and **Table 2**, respectively.

Tioguanine tolerability

In 37 (80%) of the 46 patients, TG was well tolerated until the last clinical evaluation within the study period. The median TG treatment duration was 22.4 months (range 0.3-72.8). Two patients had a follow-up of less than six months and three patients failed TG therapy within six months due to intolerability. Of the remaining 41 patients who tolerated TG for at least six months, a further six patients failed TG therapy, three of whom were therapy refractory and underwent colectomy and three who were intolerant. **Table 3** summarizes the characteristics of the patients who failed TG therapy and a Kaplan-Meier curve is depicted in **figure 1**.

Two out of the four patients who were initially resistant to AZA/MP did not tolerate TG. One developed arthralgia and myalgia within two weeks, and the other developed thrombocytopenia within four months of TG therapy, respectively. Adverse events, similar to those that had been experienced with AZA, recurred in one of the six patients intolerant of TG. Pancreatitis, observed in one patient during AZA therapy, did not recur with TG therapy. Cox regression analysis revealed that the concomitant use of aminosalicylates was the only independent variable that protected patients against TG withdrawal due to adverse events (Hazard ratio 0.11, 95% C.I. 0.03-0.48).

Table 1. Baseline characteristics (n=46).

Sex (M/F)	23 / 23
Age (y) (median, range)	38 (19-71)
Location UC (n, %)	
Proctitis	16 (35)
Left-sided	17 (37)
Pancolitis	9 (20)
Unknown	4 (8)
Duration of disease (months) (median, range)	52 (2-259)
Thiopurine pre-treatment (n, %)	
Azathioprine	46 (100)
Mercaptopurine	2 (4)
Both	2 (4)
Other drug history (n, %)	
Aminosalicylates	43 (93)
Corticosteroids	43 (93)
Anti-TNF	3 (7)
Methotrexate	2 (4)
Cyclosporine	4 (9)
Reasons for TG initiation (n, %)	
AZA/MP intolerance	**42 (91%)**
Gastrointestinal complaints	16 (35)
Rash	4 (9)
Arthralgia or myalgia	13 (28)
Fever	11 (24)
Liver test abnormalities	4 (9)
Pancreatitis	1 (2)
Headache	2 (4)
AZA / MP resistance	4 (9)
TG dose (mg/day) (median, range)	20 (18-24)

Table 2. Co-treatments at any time during TG therapy.

Drug	**n (%)**
5-Aminosalicylates	36 (78%)
Corticosteroids	38 (83%)
Cyclosporine	7 (15%)
Infliximab	7 (15%)
Tacrolimus	1 (2%)

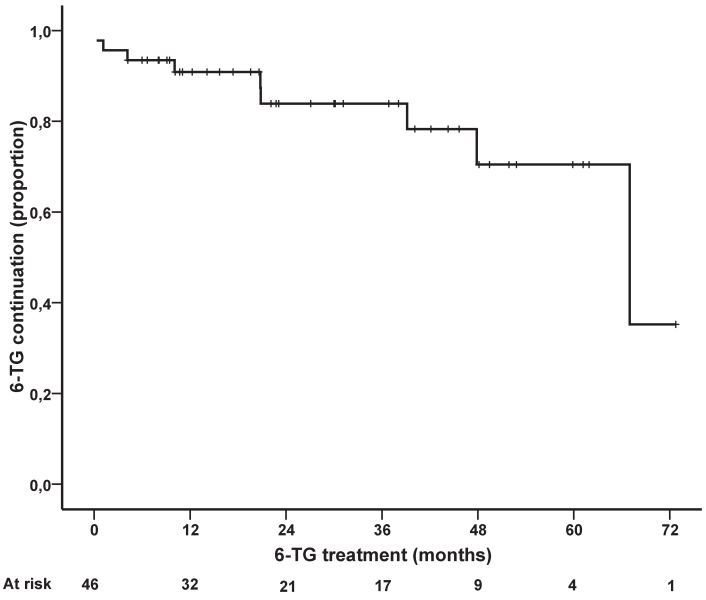

Figure 1. Kaplan-Meier curve showing TG therapy (dis-)continuation. In nine out of 46 patients TG was withdrawn due to intolerance (n=6) and resistance (n=3), as pointed out in **Table 3**.

Table 3. Patient characteristics of all who failed TG therapy.

Patient	Sex (M/F)	Age (y)	Duration disease (months)	Reason stop TG	Duration TG (months)	Reason stop AZA or MP
1	F	37	172	Arthralgia/myalgia	0.3	Resistance to AZA
2	F	22	2	Gastrointestinal complaints	2	Gastrointestinal complaints on AZA
3	M	36	81	Thrombocytopenia + liver test abnormalities	4	Resistance to AZA
4	M	38	222	Gastrointestinal complaints	10	Arthralgia on AZA
5	M	36	9	Thrombocytopenia + liver test abnormalities	21	Arthralgia + fever on AZA
6	F	30	8	Fatigue	21	Gastrointestinal complaints on AZA
7	M	45	10	Refractory*	39	Arthralgia + fever on AZA
8	F	30	54	Refractory*	48	Gastrointestinal complaints on AZA
9	M	56	32	Refractory*	67	Fever on AZA

* Surgical intervention indicated

Safety

Overall, laboratory findings did not markedly change during TG treatment (**Table 4**). The median total white blood cell count decreased from 9.0x10^9/L (range 3.1-18.5) to 7.0x10^9/L (range 3.5-21.7) (P<.001). The median 6-TGN concentration during follow-up (n=10)

was 278 pmol/8x10^8 RBC (range 68-492). Twenty-one patients (46%) had undergone an abdominal ultrasonography after a median TG treatment duration of 16 months (range 9-41). Seventeen patients had no abnormalities in the liver or spleen. Liver abnormalities were observed in four patients. Two patients had mild steatosis, one had a hemangioma, and one had hepatomegaly. Laboratory findings of this latter patient revealed no abnormalities. In particular there was no thrombocytopenia or liver test abnormalities. Liver histology however revealed sinusoidal dilatation.

Liver biopsy was performed in 12 patients (26%) after a median TG treatment of 36 months (range 10-44). None of these specimens showed NRH. Steatosis was found in one (8%), fibrosis in one (8%), sinusoidal dilatation in two (17%), non-specific regeneration in four (33%) and normal liver histology in four biopsies (33%). **Table 5** shows laboratory results obtained at time of the liver biopsy with the corresponding liver histology. Liver biopsy was not performed in the two patients withdrawing from TG treatment due to thrombocytopenia.

Table 4. Laboratory results at baseline compared with those assessed at the last evaluation (median and ranges) after a median TG treatment duration of 20 months (range 7-68).

Parameter	Baseline	Follow-up
Hemoglobin (mmol/l)	8.3 (5.8-9.6)	8.5 (6.0-10.0)
WBC (x10*9/l)	9.1 (5.4-18.5)	7.0 (3.5-21.7)*
Platelet count (x10*9/l)	299 (195-489)	275 (78-510)
ALAT (U/l)	23 (12-95)	13 (8-64)
ASAT (U/l)	19 (17-139)	20 (13-50)
GGT (U/l)	22 (8-469)	22 (9-174)
AP (U/l)	60 (45-620)	66 (34-114)
Bilirubin (μmol/l)	8 (6-129)	7 (4-23)

WBC, white blood cell count; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; GGT, gamma glutamyltransferase; AP, alkaline phosphatase. * P<.001

Table 5. Laboratory results at time of liver biopsy (n=12). Data are displayed in median with ranges. The median TG treatment duration at the time of liver biopsy is 36 months (10-44).

	Normal; n=4	Steatosis; n=1	Fibrosis; n=1	Sinus dilatation; n=2	Aspecific regeneration; n=4	P
ASAT (U/l)	21 (17-25)	47 (-)	(-) (-)	26 (18-33)	17 (16-22)	.158
ALAT (U/l)	11 (-)	52 (-)	38 (-)	19 (-)	15 (9-20)	.652
GGT (U/l)	9 (-)	174 (-)	27 (-)	31 (-)	17 (9-24)	.417
Alkaline phosphatase (U/l)	61 (52-70)	66 (-)	66 (-)	83 (76-90)	47 (43-51)	.078
Bilirubin (μmol/l)	-	-	-	6 (-)	6 (5-7)	.838
Hemoglobin (mmol/l)	8.3 (8.0-90)	9.0 (-)	9.2 (-)	9.0 (-)	7.6 (7.0-10.0)	.295
Platelet count (x10*9/l)	312 (311-312)	226 (-)	305 (-)	221 (177-264)	298 (207-390)	.530
White blood cell count (10*9/l)	6.8 (5.0-9.0)	6.3 (-)	8.4 (-)	7.0 (-)	6.1 (5.0-7.0)	.539

Endoscopic remission

Baseline and follow up endoscopy reports were available for 10 patients (22%). At baseline, only one out of 10 revealed endoscopic remission. At follow-up, after a median treatment duration of 14 months (7-26), four out of ten revealed endoscopic remission (p=.180). These four patients did not include the patient with endoscopic remission at baseline. Logistic regression analysis could not substantiate the effect of co-treatments on these endoscopic findings.

DISCUSSION

Since the initially promising results regarding the use of TG in IBD, several other relatively small studies have evaluated the safety and efficacy of TG in IBD treatment. Most of these studies included predominantly CD patients. The aim of the present study was to evaluate the tolerability, safety and efficacy of TG in UC patients who had previously failed or been intolerant of conventional thiopurine therapy. Tioguanine was well tolerated in the majority of this selected and difficult to treat population. In addition, TG is relatively safe in the long-term; no NRH was detected by liver biopsy. Endoscopic data from this study suggest that TG is effective, although statistically this could not be substantiated. The greatest strength of this study is its long-term follow-up, which is up to eight times longer than previously reported[14,15]. In addition, our study included a total of 46 UC patients, the largest cohort so far.

With a median treatment duration of almost two years, a daily dose of approximately 20mg TG was well tolerated in 80 percent of UC patients in our cohort. Within this relatively large group, only nine patients failed TG treatment. Three patients underwent colectomy after more than three years of successful TG use, whereas six patients withdrew therapy due to adverse events. The low attrition rate observed in our study reflects the favorable tolerability and safety profile of TG.

In contrast, a recent study from Sweden evaluated TG in CD patients and showed that TG was not tolerated in 43 percent of patients[16]. We speculate that the large proportion of patients intolerant of TG in that study may have been due to the relatively high daily dose of 40mg, notwithstanding the fact that others observed good tolerability with the same dose[9,17]. Adverse events occurring during TG therapy tend to be different from those experienced during AZA or MP therapy. This may be expected due to the differences in drug metabolism. Accordingly, only one out of six patients intolerant of TG experienced adverse events similar to those that had occurred during conventional thiopurine therapy. Interestingly, as we observed in one of our patients, thiopurine-induced pancreatitis usually does not recur with TG therapy[17,18].

In the present study, two patients (4%) withdrew from TG therapy due to a combination of liver test abnormalities and thrombocytopenia, which occurred after only four months in one and after 21 months in the other. It is worth noting that the patient who developed these laboratory abnormalities within four months of therapy, already had a reduced platelet count of 126 x 10^9/L without liver test abnormalities at the time of TG initiation, suggesting that previous thiopurine therapy may have initiated this toxicity.

In this study, the concomitant use of aminosalicylates protected against the failure of TG treatment due to adverse events. This may be related to the fact that aminosalicylates can inhibit the enzyme thiopurine S-methyl transferase (TPMT), which drives the conversion of TG towards the putative pharmacologically inactive, but potentially toxic, compound methylthioguanine (MeTG)[19]. In theory, inhibition of TPMT causes a reduction in MeTG and an increase in 6-TGN concentrations. A dose-dependent increase in 6-TGN concentrations was indeed observed in IBD patients using aminosalicylates in combination with AZA or MP[20]. Based upon these findings, we speculate that TG-associated adverse events are prevented by reduced MeTG concentrations through the concomitant use of aminosalicylates. Further investigation is warranted to corroborate this.

Safety concerns have largely discouraged the use of TG in IBD treatment. In particular, TG therapy was related to a high incidence of NRH, a histological abnormality of the liver which in severe cases may give rise to portal hypertension[6]. Of note, the background rate of NRH in a control population is about two to three percent[21,22] and NRH has also been observed in thiopurine naïve IBD patients[23]. Ever since the association of NRH with TG only a few studies have been carried out using TG in IBD. As the frequency of NRH differed largely between these studies, a dose-dependent effect was presumed[7,24,25]. Accordingly, a European consensus was established regarding the further use of TG in IBD treatment, and included recommendations with regard to target dosages and the performance of ultrasonography and liver biopsy during follow-up[13].

In the present study, upper abdominal ultrasonography was performed in 21 patients and revealed no abnormalities in the majority. In one patient hepatomegaly was observed. However, in the absence of abnormal liver tests or platelet count in this patient, findings which make the presence of NRH unlikely, no liver biopsy was performed. Nodular regenerative hyperplasia was not observed in any of the obtained liver biopsies. This may have been due to a less toxic dosing regimen. Non-specific regeneration was observed in several patients, the relevance of which remains unknown but appears clinically insignificant. Importantly, no laboratory abnormalities were present in these patients. Yet, in the two patients with a combination of thrombocytopenia and liver test abnormalities NRH can not be ruled out with absolute certainty as no liver histology was obtained. Although we do not have any information about the clinical course of these patients after TG withdrawal, it has previously been shown that thiopurine associated NRH with concomitant raised portal pressures may ameliorate after withdrawing these drugs[26,27]. Since ultrasonography and liver biopsy were performed in only a small proportion of patients, selection bias is very likely to be present. Although ultrasonography is unlikely to be of as much value in diagnosing NRH as MRI techniques, in some studies MRI has been shown to have a limited sensitivity and specificity regarding the diagnosis of NRH[7,28]. Ultrasonography may identify signs that are secondary to NRH, such as hepatosplenomegaly and collateral veins. With regard to the liver biopsies, we believe that the reported frequency of histological abnormalities overestimates the true prevalence in TG using patients, as a liver biopsy is more likely to be performed in patients with liver test abnormalities than in patients without such laboratory abnormalities.

Due to the limited data available, we can not draw any firm conclusions regarding the efficacy of TG therapy in UC treatment. In our study, including a relatively high number of patients with proctitis, we did observe a higher proportion of patients with endoscopic remission during follow-up as compared to baseline, although this difference was not statistically significant. Comparable results were reported by Teml and colleagues, who evaluated endoscopic findings at baseline and after a 26 week course of TG in 10 UC patients. They showed that endoscopic findings were significantly improved after TG treatment[14]. Some other small prospective trials evaluated the disease activity and showed promising results[15,18,29]. However, none of these were controlled trials.

In conclusion, our study showed that TG is well tolerated in UC patients previously intolerant of or resistant to conventional thiopurines, even in the longer term. In addition, its use seemed safe, although careful monitoring of laboratory values remains mandatory. Even though the efficacy of TG could not be accurately determined in this study, the overall results may support the use of TG as a rescue drug in UC treatment. Well-designed prospective trials are required to further evaluate TG efficacy.

ACKNOWLEDGEMENT

This study was supported by an unrestricted grant from HLW pharma bv, Helmond, the Netherlands. We owe special thanks to Anne Dahele for critically reviewing the paper.

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